

사람 정상 코점막 상피세포에서 섬모세포의 표식자로서 MUC8에 관한 연구

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MUC8 as a Ciliated Cell Marker in Human Nasal Epithelium

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ABSTRACT

Background and Objectives : To examine the MUC8 mRNA expression patterns according to the mucociliary differentiation of the normal human nasal epithelial (NHNE) cells, and to investigate the localization of the MUC8 proteins in the nasal polyps. **Materials and Methods** : The passage-2 NHNE cells were cultured using an air-liquid interface technique and nasal polyp specimens. On the 2, 7, 14, and 28 days after confluence, the ciliated cells were counted using cytospin slide immunostaining using H6C5 and β -tubulin, and the MUC8 mRNA levels were determined using real-time quantitative PCR. After synthesizing the polyclonal anti-MUC8 peptide antibodies, MUC8 immunostaining was preformed using the nasal polyps. The MUC8 mRNA and protein levels were determined with the NHNE cells treated with IL-1 β (10 ng/ml for 24 hours) using RT-PCR and Western blot analysis. **Results** : The increasing pattern of the number of ciliated cells as well as the MUC8 gene expression level with increasing culture time in the NHNE cells was quite similar. MUC8 was expressed in the ciliated cells of the human nasal polyps. The MUC8 protein level as well as the mRNA level was up-regulated as a result of the IL-1 β treatment. **Conclusions** : This study indicates that the MUC8 protein is expressed in ciliated cells from the human nasal epithelial cells and is up-regulated by the IL-1 β treatment. These results suggest that the MUC8 gene and protein expression levels might be used as a ciliated cell marker in the human nasal epithelium. (Korean J Otolaryngol 2005;48:455-9)

KEY WORDS : MUC8 · Ciliated cell · Nasal epithelial cell.

(mucus) .
.
.
1)
197가
MUC1, MUC2, MUC3, MUC4, MUC5AC, MUC5B,
MUC6, MUC7, MUC8,¹⁾ MUC9,²⁾ MUC11, MUC12,
MUC13, MUC15, MUC16, MUC17, MUC18, MUC19³⁾
MUC20 . 가 MUC5AC MUC5B
(gel)
4)5) MUC5AC가
6)
MUC-
7)
5AC mRNA
MUC1, MUC4, MUC6, MUC7, MUC8, MUC13

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MUC8 mRNA
가
8)
가 MUC8 mRNA
MUC8 mRNA
MUC8
, MUC8
가
MUC8 mRNA
MUC8 (localization)
, 가 MUC8 IL1 -
mRNA
Air - liquid interface(ALI)
10⁵ (passage - 2)
(Transwell - clear, Costar Co., Cambridge, MA)
0.5 ml
basal epithelial growth medium(BEGM)
Dulbecco 's modified Eagle 's medium(DMEM)
1 : 1 11) , 9
,
. 9
ALI
. MUC8 mRNA
2 , 7 , 14
28 RNA
cytospin 가
(H6C5 ; 1 : 1000, University of North Carolina, NC, Dr. Davis CW) - tubulin
(Sigma, St. Louis, MO)
H6C5 tubulin sl-
ide 1000 , Stu-
dent t - test .

Real - time quantitative PCR

Primer probe PE Biosystems Perkin

Elmer Primer Express software

, Commercial reagents(TaqMan PCR Universal PCR Master Mix : PE Biosystems, Foster City, CA)
protocol . 25 µl
1 µl cDNA(reverse transcription) 800 nM
가 primer, 200 nM TaqMan
hybridization probe가 . real - time PCR probe
5 'end carboxyfluorescein(FAM)
, 3 'end quencher carboxytetramethylrhodamine(TAMRA)
primer Taq-
Man probe가 .
: MUC8, forward 5 ' - GACCTGCCCCCATGGAC - 3 '
reverse 5 ' - CAGGAGTTTCGAGACCAGCCT - 3 '
Taqman probe 6FAM - CCACCTCCGAGCCCGTCACT-
GAG - TAMRA. - 2microglobulin(2M) forward 5 ' -
CGCTCCGTGGCCTTAGC - 3 ' reverse 5 ' - GAGT-
ACGCTGGATAGCCTCCA - 3 ' Taqman probe
6FAM - TGCTCGCGCTACTCTCTCTTTCTGGC - TA-
MRA가 . Real - time RT - PCR PE Biosys-
tems ABI PRISM 7700 Sequence Detection System
(Foster City, CA) , thermocycler
(ABI PRISM 7700 Sequence Detection System) para-
meters 50 2 , 95 10 , 95
15 , 60 1 40 cycle
, MUC8 mRNA
comparative cycle of the threshold(CT) method
, - 2M .

MUC8 peptide

KTSCPRPLQEGTPGS sequence 가 15 - mer pep-
tide가 12) glutaraldehyde keyhole
limpet hemocyanin(KLH) . KLH - peptide
가

MUC8

24 4% paraformaldehyde
12% 18% sucrose
. insert 10 µm frozen section
MUC8
peroxidase anti - rabbit
rabbit IgG .

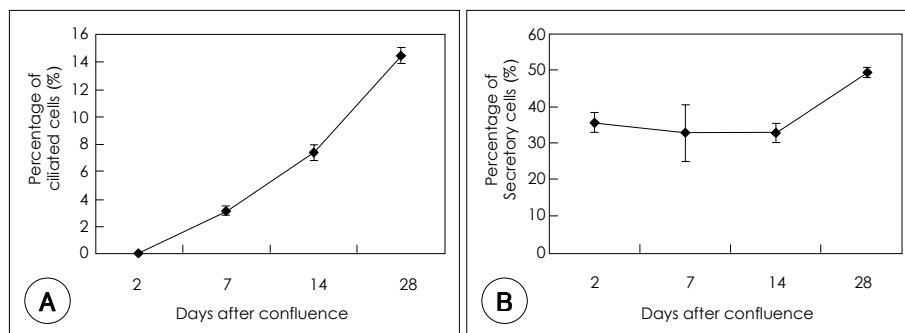


Fig. 1. Percentage of ciliated and secretory cells according to the culture duration in NHNE (Normal human nasal epithelial) cells. The number of ciliated cells increased as a function of differentiation (A), but the number of secretory cells remain relatively constant (B). These figures are representative of three separate experiments.

IL - 1
(passage 2)가 6 well plate
. ALI가 2
24 IL - 1 (10 ng/ml)
, RNA MUC8 RT - PCR Wes-
tern blot analysis .

IL - 1 MUC8
RT - PCR
Oligonucleotide primers
. 8) 2M oligonucleotide amplimers
RT - PCR , Clontech Laboratories(Palo
Alto, CA ; they generated a 335 - bp PCR fragment)
. RT - PCR Perkin - Elmer Cetus DNA
Thermal Cycler(Perkin - Elmer, Norwalk, CT)
, RNA(1 µg/20 µl
reaction volume) random hexanucleotide primers
Moloney murine leukemia virus RT cDNA
. mRNA
(Comparative kinetic analysis)
. PCR 50 ng/mL ethidium bromide
2% Seakem agarose gel(FMC, Rockland, ME)
55
(amplified products) mRNA
genomic DNA
(reverse transcriptase)
PCR
(negative control) .

IL - 1 MUC8
Western
IL - 1 2x lysis buffer[250 mM Tris -
Cl(pH 6.5), 2% SDS, 4% - mercaptoethanol, 0.02% BPB,

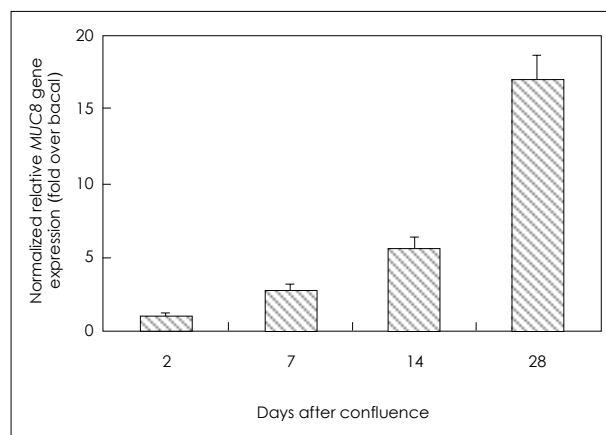


Fig. 2. MUC8 mRNA expression level according to the culture duration in NHNE (normal human nasal epithelial) cells using real-time quantitative PCR. MUC8 mRNA expression increased as a function of differentiation and its increasing pattern was similar to that of the ciliated cell number.

10% glycerol] cell lysate 6%
SDS - PAGE polyvinylidene difluo-
ride membrane(PVDF ; Millipore, Bedford, MA)
. PVDF 2 Tris - buffered sa-
line[50 mM Tris - Cl(pH 7.5), 150 mM NaCl] 5%
skim milk MUC8
, TTBS anti - rabbit
(Cell Signaling Tech., Beverly, MA)
45 ECL system(Amersham -
Pharmacia, Piscataway, NJ)

MUC8

(retinoic acid)

- tubulin

2

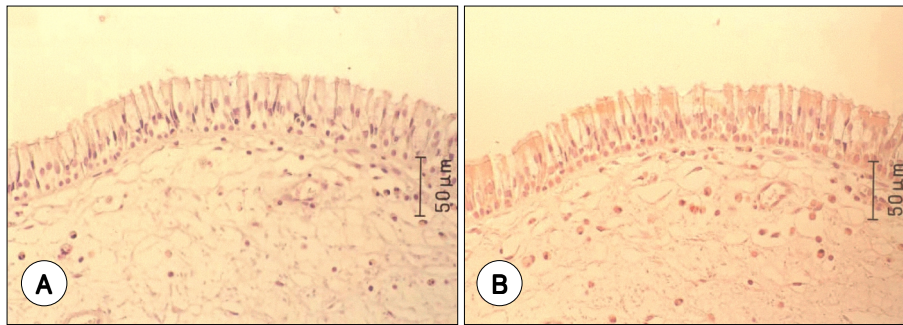


Fig. 3. Immunostaining of the polyp using polyclonal anti-MUC8 peptide antibodies ($\times 200$). Positive immunoreactivity was identified in the ciliated cells (stained dark brown) (B). Negative control showed no immunoreactivity (A).

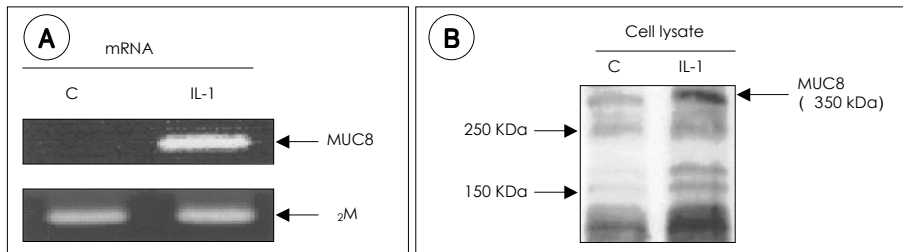


Fig. 4. RT-PCR and Western blot analysis for MUC8 in IL-1 treated (10 ng/ml for 24 hours) NHNE cells. The MUC8 mRNA expression level was up-regulated by the IL-1 treatment (A) and MUC8 protein expression level was also up-regulated by IL-1 (B). The size of the MUC8 protein was approximately 350 kDa.

7 3.1 ± 1

0.2%, 14 7.4 ± 0.5%, 28 (Fig. 3B). MUC8 cDNA

14.5 ± 0.6% (Fig. 1A). , H6C5 9.5 kb (data not shown), MUC8

2 35.6 ± 2.8%, 7 350 kDa

32.8 ± 7.8%, 14 32.8 ± 2.5% band가 350 kDa band IL -

28 1

49.4 ± 1.4% 가 (Fig. 1B).

MUC8

가 (Fig. 2).

MUC8 가

MUC8

가

MUC8

가 ,⁹⁾¹⁰⁾

anti - MUC8 peptide 가⁸⁾

(Fig. 2A)

(Fig. 2B).

cDNA (323 amino acids)

, cDNA

MUC8

가

IL - 1 MUC8 mRNA

가 , MUC8 mRNA 가

MUC8 mRNA IL - 1

가 MUC8

⁹⁾¹⁰⁾

(Fig. 3A),

anti - MUC8 peptide Western blot IL -

anti - MUC8 peptide

, MUC8 가

(Fig. 3B). Lopez - Ferrer A MUC8
(bronchial epithelium)
,¹³⁾
MUC8 peptide
Lopez

microarray 2 - D PAGE high - throu-
ghput analysis mRNA 가
(¹⁴⁾¹⁵⁾ 가
(post - transcriptional regulation)
(regulated gene)
(transcriptional regulation)

MUC8 IL - 1

IL - 1 MUC8 mRNA
가 가 MUC8

IL - 1 MUC8 가
ERK MAP kinase/RSK1/
CREB cascade pathway 가
¹⁶⁾ MUC8 cDNA promoter
, cDNA pro-
moter
MUC8

MUC8 IL - 1
가 MUC8
가 , MUC8
(marker)
: 8 .

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